The Two-Hit Hypothesis Meets Epigenetics

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The landmark paper by Kane and colleagues was the first report of DNA methylation in the promoter of the human *MLH1* gene in sporadic colon cancers with mismatch repair (MMR) deficiency. In both cell lines and primary tumors, promoter methylation was associated with loss of MLH1 protein expression and with a lack of mutations in the *MLH1* coding region. Together with subsequent papers that showed that this methylation was directly responsible for loss of MLH1 expression and MMR deficiency, the observation expanded the two-hit hypothesis of tumor suppressor gene loss in cancer to include both genetic and epigenetic mechanisms of gene inactivation. More broadly, the paper contributed to normalization of the hypothesis of an epigenetic basis for cancer development.

See related article by Kane and colleagues, Cancer Res 1997; 57:808–11

Cancer: Genetic or Epigenetic Disease?

The idea of cancer as a disease of epigenetic regulation was proposed in the 1960's based on unexpected reversal of the cancer phenotype when malignant cells undergo epigenetic reprogramming though embryogenesis (1). Epigenetic mechanisms were still mysterious at the time, and in the absence of a molecular explanation, the concept was rapidly forgotten. By the 1970's, studies of familial cancer incidence had firmly established a genetic basis for inherited cancer, and it was hypothesized that sporadic cancers would follow the same path. Knudson formulated the two-hit hypothesis to explain familial versus sporadic cancer epidemiology, proposing that the same genes are involved in both cases (2). This was confirmed molecularly with studies on familial cancer genes such as *TP53* (3). The dogma evolved to a model whereby the two hits are mutations or deletions in the same tumor suppressor gene (TSG; **Fig. 1**); familial cases would be explained by an inherited hit followed by a sporadic hit, while nonfamilial cases are caused by two rare sporadic hits, explaining the delayed age of onset when compared with familial cancers (2).

Inherited mutations in *MLH1* cause the Lynch syndrome, a familiar colon cancer predisposition disease (3). Biallelic inactivation of *MLH1* causes mismatch repair (MMR) deficiency, accumulation of mutations, and eventual cancer formation. The phenotype of MMR deficiency was known before the genotype was elucidated (it was termed microsatellite instability or MSI at the time), and it was clear early on that MMR defects were present in both familial and sporadic colon cancers (4). In accordance with Knudson's hypotheses, it was expected that the genes that cause familial versus sporadic MMR-deficient cancers would be the same. Kane and colleagues tried to confirm this (4). Unexpectedly, while they found that sporadic MSI+ cases had loss of MLH1 protein, they could find no coding sequence mutations in this gene, challenging the prevailing notion at the time.

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In the 1980's, the unorthodox idea of an epigenetic basis for cancer resurfaced, powered by an actual proposed mechanism—permanent silencing of TSG expression by promoter DNA methylation (5). As besets many "out-of-the-box" hypotheses, this one was met with considerable early skepticism. Nevertheless, after Kane and colleagues observed unexplained loss of MLH1 expression in colon cancers, they went on to show that those sporadic cell lines and primary tumors that lacked MLH1 expression also had dense DNA methylation in a CpG rich area in the gene promoter (4). This observation was therefore consistent with Knudson's tenets, but with a twist—the gene in sporadic and familial cancers was the same (*MLH1*) but the mechanism of inactivation was different (DNA methylation vs. mutations; **Fig. 1**). The fact that *MLH1* was a *bona fide* cancer predisposition gene (a "gatekeeper" in Vogelstein's model; ref. 3) inactivated by DNA methylation was a turning point in the wide acceptance of the epigenetic basis of cancer hypothesis. It was further buttressed by the demonstration that the MMR phenotype can be reversed by removal of DNA methylation (6) and also by the identification of other TSGs with similar genetic/epigenetic mechanisms of inactivation (5). As can be seen in cancer biology textbooks 25 years later, the paper by Kane and colleagues contributed to rewriting the chapters on the mechanistic basis of cancer development—cancer is now seen as a genetic and an epigenetic disease.

The Two-Hit Hypothesis Revised

An important consequence of the paper by Kane and colleagues (and other papers at the time) was the modification of the two-hit hypothesis to include both genetic and epigenetic mechanisms of gene inactivation (**Fig. 1**). Because epigenetic changes are reset during embryogenesis, it is not surprising that, with rare (and fascinating) exceptions, all familial cancers are caused by genetic changes. For reasons not yet fully understood, some TSGs are resistant to epigenetic inactivation. In those cases, the second hit in familial cancers as well as both hits in sporadic cancers are also genetic. For the other TSGs (such as *MLH1*, *VHL*, *CDKN2A*, *BRCA1*, and others), the second hit in familial cases can be either genetic or epigenetic, and both hits in sporadic cases can similarly be either genetic or epigenetic. Lynch syndrome provides an interesting study in this genetic/epigenetic duality. The *MSH2* gene is a frequent cause of familial disease but is almost never epigenetically inactivated, and it is very rarely responsible for sporadic colon cancers. By contrast, the *MLH1* gene is also a frequent cause of familial disease, but it is susceptible to epigenetic

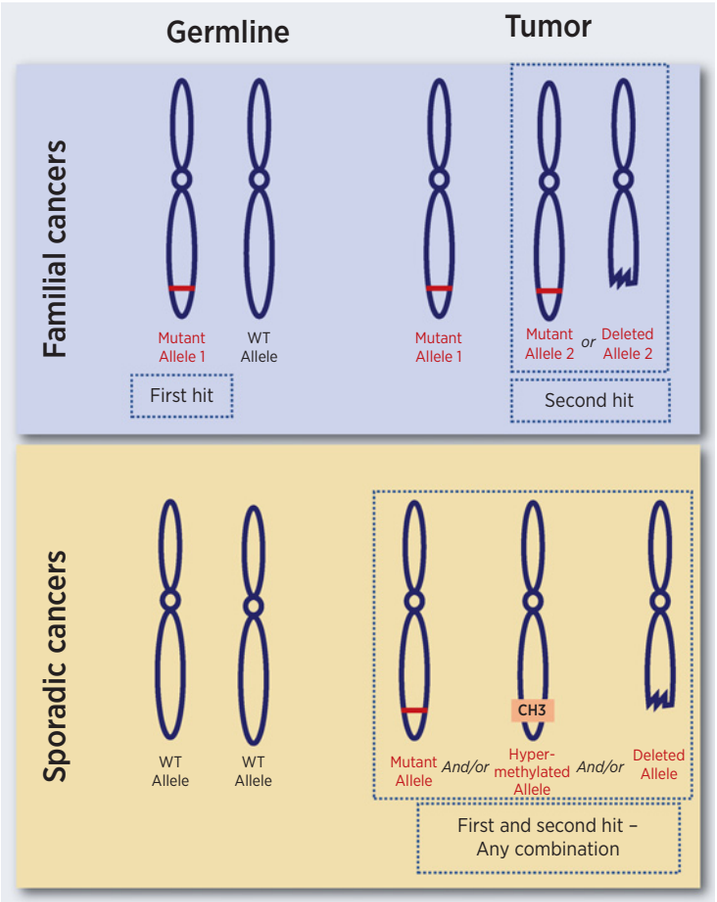

Figure 1.
The two-hit hypothesis meets epigenetics. By the 1990's, Knudson's two-hit hypothesis had evolved to postulate that familial cancer predisposition is due to a germline mutation in a TSG (top left), while actual cancer development follows a sporadic mutation (or deletion) in the second allele (top right). It also postulated that sporadic cancers of the same tissue type were due to two acquired hits (mutation or deletion) in the same TSG (bottom). The paper by Kane and colleagues (and other papers in that period) revised that model for sporadic cancers to include epigenetic inactivation by promoter DNA hypermethylation as either the first hit, the second hit, or both (bottom right). The figure is a simplification; subsequent data showed that in very rare cases familial cancers can be caused by germline hypermethylation, that the second hit in familial cancers can also be DNA hypermethylation, and, in the case of *MLH1* in colon cancer, the "hits" in sporadic cases are almost always hypermethylation caused by the CpG island methylator phenotype.

inactivation and is almost always responsible for MMR-deficient sporadic colon cancers.

Cycles of Instability

In addition to revising the two-hit hypothesis, the paper by Kane and colleagues suggested that epigenetic changes can trigger genetic changes by inducing DNA repair defects. In an interesting additional twist, *MLH1* methylation in sporadic cancers was subsequently shown to be caused by a broader epigenetic deregulation, the CpG island methylator phenotype (CIMP; ref. 7). CIMP+ cancers inactivate multiple TSGs simultaneously, and this phenotype of epigenetic instability has been described in almost all cancer cell types (7). Intriguingly, *MLH1* is a common CIMP target in colon and endometrial cancers, but not in other CIMP+ cases. In acute myelogenous leukemia (AML) and in glioblastoma multiforme (GBM), CIMP is caused by genetic defects in the TCA cycle, namely mutations in *IDH1* or *IDH2* (8). These mutations lead to accumulation of metabolites that inactivate the TET enzymes, which are responsible for protection against DNA methylation in CpG islands (8). Thus, mutations in metabolic regulation genes can lead to epigenetic instability. In turn, epigenetic instability can lead to genetic instability by inducing DNA repair defects (7). As one can imagine, these cycles of instability can be repeated many times in the lifetime of a cancer, contributing to progression, metastasis, and therapeutic resistance.

Unlike AML and GBM, mutations in TCA cycle genes have not been commonly seen in CIMP+ colon cancers (9) or gastric cancers. Even in AML, CIMP can only be explained by mutations in about half the cases. The etiology of CIMP in mutation-negative cases remains speculative. There is a strong association between CIMP and Epstein-Barr virus in gastric cancers (7), and an intriguing link between CIMP and fusobacterium in colon cancers (9), suggesting

an environmental etiology to CIMP in some cases. It is possible for example that infections lead to metabolic deregulation, disrupting TET function and leading to DNA hypermethylation.

Clinical Implications

The diagnosis of a familial cancer syndrome can save lives through early screening and intervention. Lynch syndrome has an incomplete penetrance and can first manifest at an advanced age. While colon cancers are routinely screened for MMR defects, a positive result does not always trigger an investigation of germline defects because the frequency of sporadic MMR-deficient cancers is higher than that of familial MMR-deficient cancers after the age of 60. The paper by Kane and colleagues (and subsequent confirmation) suggests that *MLH1* methylation can serve as a quick additional screen in this respect. MMR-deficient colon cancers that lack MLH1 expression but have no *MLH1* promoter DNA methylation should trigger a very high index of suspicion for Lynch syndrome.

Finally, it is worth noting that epigenetic changes mediated by DNA methylation are potentially reversible through the use of drugs that target DNA methyltransferases (10). The paper by Kane and colleagues (4) and other papers showing the importance of DNA methylation in TSG regulation in cancer (5) triggered a revival in interest in these drugs, eventually leading to their FDA approval and broad usage in patients with myeloid malignancies (10). While their activity in solid tumors remains limited, there continues to be interest in developing DNA methylation–based treatment or prevention strategies that could be especially useful in cases with CIMP and *MLH1* promoter DNA methylation.

Conclusions

The paper by Kane and colleagues had a straightforward message: MLH1 expression was missing in MMR-deficient sporadic colon cancers and this was associated with promoter DNA methylation rather than coding mutations in the gene (4). This deceptively simple message had a profound influence in that it lent legitimacy to the hypothesis of DNA methylation–mediated TSG inactivation in cancer. It helped rewrite textbooks—"Cancer is a genetic ***and*** an epigenetic disease."

Authors' Disclosures

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